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(54) Conjugates of epitopes of HIV with a protein complex from Neisseria

(57) Antigenic conjugate of the formula

$(SPNE)_n$ - (OMPC)

wherein SPNE is a selected principal neutralisation epitope of HIV (as defined in Table A), or a fragment thereof of at least five amino acids, which incorporates the sequence DRW or DKW;

OMPC is the purified outer membrane proteosome of Neisseria, preferably N. meningitidis;

n is from 1 to 200;

may be used in vaccines. The conjugate may be substituted by anion(s), and conjugation may be via a bigeneric spacer.

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TITLE OF THE INVENTION

NEUTRALISATION EPITOPES

BACKGROUND OF THE INVENTION

Acquired Immune Deficiency Syndrome (AIDS) is the clinical manifestation of the apparent infection of CD4 helper T-cells and other cell targets by human immunodeficiency virus (HIV), also previously referred to as human T-lymphotropic virus type III (HTLV-III), Lymphoadenopathy-associated virus (LAV), or AIDS-related virus (ARV) (hereinafter collectively "HIV"). AIDS is a transmissible deficiency of cellular immunity characterized by opportunistic infections and certain malignancies. A similar disease, AIDS-related complex (ARC), shares many of the epidemiological features and immune abnormalities with AIDS, and often precedes the clinical manifestations of AIDS.

AIDS is a disease of a virus with a unique collection of attributes. HIV itself targets the immune system; it possesses a reverse transcriptase capable of turning out highly mutated progeny; it is sequestered from the immune system and it has hypervariable sequences in the (env) region. See, e.g., Hilleman, M.R., Vaccine 6, 175 (1988); Barnes, D.M., Science 240, 719 (1988).

One consequence of these attributes is the diversity of HIV serotypes. The principal neutralizing determinant is an epitope residing in a hypervariable region of the (env) region. As a result, neutralizing antibodies directed against this epitope are generally extremely type-specific; that is, they neutralize only the parental virus and not other variants. Appropriate immunological therapies for AIDS require

special consideration of this serological diversity. In particular, it is widely believed that a likely AIDS vaccine will be polyvalent and comprise HIV determinants corresponding to each serotype.

Elicitation of neutralizing antibody is now regarded as one of the key consequential features in the successful design of an HIV immunological therapy. When a virus-specific antibody neutralizes its virus, it blocks the replication of the virus, but the precise mechanism is not fully characterized and is thought to vary with virus and target cell. See, e.g, Dimmock, N.J., Trends in Biochem. Sci. 12, 70 (1987).

Applicants have now practiced an unusual method to make vaccines suitable for the serological diversity of HIV and the requirements of eliciting neutralizing antibody. Applicants employ a monoclonal antibody to define a broadly neutralizing response, then identify oligopeptide epitopes bound by this monoclonal antibody out of a large random array or library. The identified epitopes do not have to share any protein sequence with the native HIV protein used to generate the monoclonal antibodies in the first place.

In this invention, the screening antibody is 2F5, a commercially available, human monoclonal antibody. The antibody has been shown to recognize a sequence within the ectodomain of the viral gp41 transmembrane glycoprotein. Antibody-selection of recombinant phage expressing a random epitope library shows that the generalized binding determinant is the sequence ELDKWA. The antibody's virus-neutralizing characteristics are assessed using a panel of T-lymphoblastoid cell-adapted HIV-1 variants as well as primary HIV-1 isolates. 2F5 is capable of neutralizing a broad range of diverse virus variants. In addition, the antibody exhibits notable neutralizing activity when tested against primary isolates in human peripheral blood mononuclear cell cultures. Sequence analysis of the primary isolates shows that 2F5-mediated neutralization correlated with the presence of its putative binding site and that the sequence of this site is highly conserved when compared to the variable domains of the gp120 envelope glycoprotein.

Applicants have discovered novel homologous oligopeptides useful as neutralization epitopes specific for HIV, known hereafter as selected principal neutralization epitopes (SPNEs). These oligopeptides are of synthetic origin.

Applicants have conjugated the oligopeptides of interest to an immunological carrier to provide an immunological conjugate useful as an AIDS vaccine. Alternatively, this immunological conjugate(s) is useful for generating better and improved broadly neutralizing antibodies for HIV, which are in turn useful for passive immunization and like therapies. The SPNEs as well as their immunological conjugates are also useful as reagents in the assay of virus in a human host, and in screening blood in blood banks.

A method for screening phage epitope libraries with an antibody of desired specificity or screening antibody is also described. For this screening, applicants have developed a novel selection procedure for the selection of phages bearing epitopes that bind antibody of desired specificity. The screening method of the present invention includes such selection, and, optionally, an identification method for identifying phages bearing desired epitopes.

BRIEF DESCRIPTION OF THE INVENTION

Synthetic amino acid sequences of Table A that bind a broadly neutralizing human monoclonal antibody (2F5 antibody) specific for the HIV principal neutralization determinant are selected and identified from oligopeptide epitope libraries, and are useful in immunological conjugates with OMPC for vaccination against AIDS or ARC, as well as in the production of other HIV-specific broadly neutralizing antibodies for passive immunity against AIDS or ARC. Screening methods for selecting and/or identifying desired oligopeptide epitopes from phage epitope libraries are also described. The SPNEs and their conjugates are also useful in the detection of HIV, or antibodies to HIV in blood samples, for the purpose of screening, clinical evaluation and diagnosis.

ABBREVIATIONS AND DEFINITIONS

AIDS	Acquired immune deficiency syndrome
ARC	AIDS-related complex
conjugation	The process of covalently attaching 2 (sometimes 3) molecules each containing one or more immunological determinants, e.g., HIV envelope fragments and OMPC
conjugate	Result of conjugation, also known as an antigenic conjugate or immunological conjugate. Coconjugates are a special subgenus of conjugates.
Flanks	Flanking regions for SPNE. Such flanks are selected from either poly (gly, ser, ala, val), or, a combination of amino terminal ADGA (SEQ ID NO:37) and carboxy terminal GAAGA (SEQ ID NO:38).
HIV	Generic term for the presumed etiological agent of AIDS and/ or ARC, also referred to as strains HTLV-III, LAV, and ARV

Library	A collection of DNA or oligopeptide sequences, of defined length, with or without limited sequence restrictions
OMPC	Outer membrane proteosome
PCR	Polymerase chain reaction
poly (gly, ser, ala, val)	a linear, random polymer of amino acids selected from the group consisting of glycine, serine, alanine or valine.
Recombinant fusion polypeptide (RFP)	A polypeptide or oligopeptide expressed as a contiguous translation product from a spliced foreign DNA in a recombinant eukaryotic or procaryotic expression system, wherein the spliced foreign DNA is derived from 2 or more coding sequences of different origin, and joined together by ligation or PCR.
Recombinant protein	A polypeptide or oligopeptide expressed by foreign DNA in a recombinant eukaryotic or procaryotic expression system.

Recombinant expression system	A cell containing a foreign DNA expressing a foreign protein or a foreign oligopeptide.
SPNE	Selected Principal Neutralization Epitope, which is a principal neutralization determinant bound by one or more broadly neutralizing antibodies. SPNE is defined as including consensus sequences. SPNE may have flanks.

AMINO ACIDS

Full Name	Three-letter symbol	One-Letter symbol
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic acid	Asp	D
Asn or Asp	Asx	B
Cysteine	Cys	C
Glutamine	Gln	Q
Glutamic acid	Glu	E
Gln or Glu	Glx	Z
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P

AMINO ACIDS CONT'D.

Full Name	Three-letter symbol	One-Letter symbol
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosin	Tyr	Y
Valine	Val	V
Norlencine	Nle	
	Xaa	any amino acid

NUCLEOTIDES BASES IN DNA OR RNA

Name	One-letter symbol
Adenine	A
Cytosine	C
Guanine	G
Thymine	T
Uracil	U

The terms "protein," "peptide," "oligopeptide," and "polypeptide" and their plurals have been used interchangeably to refer to chemical compounds having amino acid sequences of five or more amino acids. "Amino acid" refers to any of the 20 common amino acids for which codons are naturally available, and are listed in the table of amino acids given above.

When any variable (e.g. SPNE) occurs more than one time in any constituent or in Formula I, its definition on each occurrence is independent of its definition at every other occurrence. Also, combinations of substituents and/or variables are permissible only if such combinations result in stable compounds.

SPNE oligopeptides may exist as peptides, as internal sequences in e.g. phage pIII proteins, in immunological conjugates with

outermembrane proteosome, or as a fragment of a recombinant fusion protein with an immunoenhancer sequence such as Hepatitis B core. The position of SPNE in a fusion protein may be N-terminal, internal or C-terminal.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides HIV selected principal neutralization epitopes of synthetic origin, immunological conjugates of these epitopes with a carrier such as OMPC, and methods of treating or preventing AIDS or ARC with these conjugates. Also described is a method of screening these epitopes from phage epitope libraries.

The epitopes of the present invention bind an HIV broadly neutralizing antibody specific for the ectodomain of HIV gp41 transmembrane glycoprotein. These epitopes were originally identified in the screening of phage epitope libraries having randomly generated epitope polypeptides accessible to the antibody. These screened polypeptides are hereinafter the selected principal neutralization epitope (SPNE) polypeptides. The sequences of these polypeptides were deduced from their corresponding DNA sequence, determined by the polymerase chain reaction. The SPNE polypeptides including consensus sequences thereof are characterized as having the sequences of Table A.

TABLE A

SEQ ID NO:1:

Asp	Lys	Asp	Lys	Trp	Ala	Ser	Leu	Asp	Ala	Arg	Thr	Gln	Met	Trp
1				5					10					15

SEQ ID NO:2:

Glu	Asn	Ala	Ile	Asp	Lys	Trp	Ala	Thr	Leu	Tyr	Trp	Ala	Tyr	Gly
1				5					10					15

SEQ ID NO:3:

Ala Phe Met Thr Val Asp Lys Trp Ala Met Phe Trp Pro Thr Gln
1 5 10 15

SEQ ID NO:4:

His Met Ser Pro Phe Asp Lys Trp Glu Phe Ile Thr Ala Arg Arg
1 5 10 15

SEQ ID NO:5:

Ser Leu Phe Ser Glu Asp Lys Trp Ser Tyr Leu His Ser Asn Ala
1 5 10 15

SEQ ID NO:6:

Met Ser His Tyr Asp Ile Asp Lys Trp Gly Gly Met Thr Ser Gln
1 5 10 15

SEQ ID NO:7:

Thr Pro Asp Gly Thr Leu Phe Phe Asp Lys Trp Ser Leu Phe Arg
1 5 10 15

SEQ ID NO:8:

Pro Lys Met Gly Ser Leu His Met Asn Asp Lys Trp Val Ser Val
1 5 10 15

SEQ ID NO:9:

Ser Leu Xaa Asp Phe Asp Lys Trp Ala Ser Leu Thr Ser Arg
1 5 10

SEQ ID NO:10:

Asp Asp Leu Asp Arg Trp Gly Arg Thr Asp Trp Asn Phe Phe Leu
1 5 10 15

SEQ ID NO:11:

Glu Asp Val Leu Asp Arg Trp Val Leu Leu Asp His Trp Asp Ser
1 5 10 15

SEQ ID NO:12:

Gly Gln Thr Ala Ala Asp Leu Asp Lys Trp Tyr Val Gly Gly Asp
1 5 10 15

SEQ ID NO:13:

Xaa Pro Leu Pro Ser Asp Leu Asp Lys Trp Glu Phe Leu Asn Arg
1 5 10 15

SEQ ID NO:14:

Val Val Asn Ser Leu Pro Leu Asp Arg Trp Ala Tyr Ser Tyr Glu
1 5 10 15

SEQ ID NO:15:

Asp Asp Leu Asp Arg Trp Xaa Xaa Leu Asp
1 5 10

SEQ ID NO:16:

Met Lys Glu Asp Asp Lys Trp Val Pro Val Lys Gly Arg Lys Phe
1 5 10 15

SEQ ID NO:17:

Met Leu Glu Gly Asp Lys Trp Ser Asp Ile Gly Met Val Phe Val
1 5 10 15

SEQ ID NO:18:

Arg Gly Arg Ala Asp Glu Pro Asp Arg Trp Ala Leu Met Phe Asp
1 5 10 15

SEQ ID NO:19:

Thr Ala Asn Ser His Trp Leu Glu Tyr Asp Arg Trp Ser Glu Val
1 5 10 15

SEQ ID NO:20:

Met Leu Glu Xaa Asp Lys Trp Ser
1 5

SEQ ID NO:21:

Met Leu Glu Xaa Asp Arg Trp Ser
1 5

SEQ ID NO:22:

Glu Leu Asp Arg Trp Ala Glu Leu Glu Met Arg Gly His Leu Asn
1 5 10 15

SEQ ID NO:23:

Glu Leu Asp Lys Trp Gly Trp Met Ala Ser His Glu Ala His Ile
1 5 10 15

SEQ ID NO:24:

Val Phe Glu Leu Asp Lys Trp Ser Gly Arg Asp Glu Glu Trp Ala
1 5 10 15

SEQ ID NO:25:

Glu Leu Asp Lys Trp Xaa Xaa Xaa Xaa Xaa Xaa Xaa Ala
1 5 10

SEQ ID NO:39:

Ser Met Leu Glu Leu Asp Lys Trp Ala Leu Leu Xaa Xaa Arg Phe Xaa Leu
1 5 10 15

SEQ ID NO:40:

Ser Met Leu Glu Leu Asp Lys Trp Ala Leu Leu Xaa Xaa Arg Phe Xaa Leu
1 5 10 15

The new SPNE amino acid sequences of this invention include any fragment thereof in the sequence listing, provided said fragment is at least five amino acids in length, and includes the DRW or DKW region.

Each SPNE amino acid sequence can be determined by DNA sequencing of phage clones amplified by the polymerase chain reaction.

The present invention also provides an effective immunogen against AIDS or ARC, and comprises an antigenic conjugate of the formula

$$(SPNE)_n\sim(OMPC) \qquad I,$$

wherein:

SPNE is the selected principal neutralization epitope of HIV, which is a polypeptide of one or more amino acid sequences, each sequence having any of sequences of Table A, or fragments thereof, said fragment having at least 5 amino acids in length and including DRW or DKW in its sequence;

n = 1-200, wherein n is the number of polypeptides of SPNE covalently linked to OMPC;

~ indicates covalent linkage;

OMPC is outer membrane proteosome of the microorganism Neisseria,

said conjugate optionally substituted with an anion or polyanion to render it soluble such as polypropionic acid, or substituted with a- which is an anion or polyanion at physiological pH, said a- consisting of one to five residues of anions selected from the group consisting of carboxylic, sulfonic, propionic or phosphonic acid, or pharmaceutically acceptable salts.

Each conjugate molecule of formula I may have different peptides conjugated thereto, or, alternatively, multiples of a single peptide species conjugated thereto, or a combination.

The antigenic conjugates of this invention are prepared by isolating, synthesizing and purifying their component parts SPNE and OMPC, then conjugating SPNE and OMPC together. Subsequent purification of conjugate mixtures may be performed as desired.

Applicants also describe a method for identifying new SPNE by the screening of phage libraries bearing randomly or semi randomly generated oligopeptide epitopes.

Polymerase Chain Reaction Amplification

Large amounts of DNA coding for SPNE protein may be obtained using polymerase chain reaction (PCR) amplification techniques as described in Mullins et al., U.S. Patent No. 4,800,159 and

other published sources. See also, for example, Innis, M.A. et al. PCR Protocols Academic Press 1990. The extension product of one primer, when hybridized to another primer, becomes a template for the synthesis of another nucleic acid molecule.

The primer template complexes act as substrate for DNA polymerase which, in performing its replication function, extends the primers. The region in common with both primer extensions, upon denaturation, serves as template for a repeated primer extension.

Taq DNA Polymerase catalyzes primer extension in the amplification process. The enzyme is a thermostable DNA polymerase isolated from Thermus aquaticus. Because it stays active through repeated elevations to high denaturation temperatures, it needs to be added only once. Deoxynucleotide triphosphates provide the building blocks for primer extension.

The nucleic acid sequence strands are heated until they separate, in the presence of oligonucleotide primers that bind to their complementary strand at a particular site on the template. This process is continued with a series of heating and cooling cycles, heating to separate strands, and cooling to reanneal and extend the sequences. More and more copies of the strands are generated as the cycle is repeated. Through amplification, the coding domain and any additional primer-encoded information such as restriction sites or translation signals (signal sequences, start codons and/or stop codons) is obtained. PCR protocols are often performed at the 100 µL scale in 0.5 ml microcentrifuge tubes. The PCR sample may be single- or double-stranded DNA or RNA. If the starting material is RNA, reverse transcriptase is used to prepare first strand cDNA prior to PCR. Typically, nanogram amounts of cloned template, up to microgram amounts of genomic DNA, or 20.000 target copies are chosen to start optimization trials.

PCR primers are oligonucleotides, typically 15 to 50 bases long, and are complementary to sequences defining the 5' ends of the complementary template strands. Non-template complementary 5' extensions may be added to primers to allow a variety of useful post

amplification operations on the PCR product without significant perturbation of the amplification itself. It is important that the two PCR primers not contain more than two bases complementary with each other, especially at their 3' ends. Internal secondary structure should be avoided in primers.

Because Taq DNA Polymerase has activity in the 37-55°C range, primer extension will occur during the annealing step and the hybrid will be stabilized. The concentrations of the primers are preferably equal in conventional PCR and, typically, are in vast excess of the template to be reproduced.

In one typical PCR protocol, each deoxynucleotide triphosphate concentration is preferably about 200 µM. The four dNTP concentrations are preferably above the estimated Km of each dNTP (10-15 µM).

Preferably PCR buffer is composed of about 50 mM potassium chloride, 10.0 mM Tris-HC1 (pH 8.3 at room temperature), 1.5 mM magnesium chloride, and 0.001% w/v gelatin. In the presence of 0.8 mM total dNTP concentration, a titration series in small increments over the 1.5-to 4-mM range will locate the magnesium concentration producing the highest yield of a specific product. Too little free magnesium will result in no PCR product and too much free magnesium may produce a variety of unwanted products.

Preferably, in a 100-µL reaction volume, 2.0 to 2.5 units of Taq DNA Polymerase are recommended. The enzyme can be added conveniently to a fresh master mix prepared for a number of reactions, thereby avoiding accuracy problems associated with adding individual 0.5-µL enzyme aliquots to each tube. A typical PCR protocol for amplification of the DNA template includes an initial 8 minute 94°C denaturation step, followed by 30 cycles of 30 seconds at 94°C (denaturation), 1 minute at 55°C (primer annealing), and 2 minutes at 72°C (polymerization). At the end of the last cycle, all strands are completed by a 5 minute incubation at 72°C.

During DNA denaturation, sufficient time must be allowed for thermal equilibration of the sample. The practical range of

effective denaturation temperatures for most samples is 92-95°C, with 94°C being the standard choice.

Primer annealing is usually performed first at 55°C, and the specificity of the product is evaluated. If unwanted bands are observed, the annealing temperature should be raised in subsequent optimization runs. While the primer annealing temperature range is often 37-55°C, it may be raised as high as the extension temperature in some cases. Merging of the primer annealing and primer extension steps results in a two-step PCR process.

Primer extension, in most applications, occurs effectively at a temperature of 72°C and seldom needs optimization. In the two-temperature PCR process the temperature range may be 65-70°C. In situations where enzyme concentration limits amplification in late cycles, the extension is preferably increased linearly with cyclic number. Usually, 25 to 45 cycles are required for extensive amplification (i.e., 1,000,000 fold) of a specific target.

Once the DNA sequence is determined, through conventional and well-known techniques, its amino acid sequence can be deduced by "translating" the DNA sequence. The resulting amino acid sequence having the selected principal neutralizing epitope of the envelope gene is then employed to synthesize large quantities of SPNE protein or fragment thereof. Synthesis is performed by organic synthesis or by recombinant expression systems, or both.

PREPARATION OF SELECTED PRINCIPAL NEUTRALIZATION EPITOPE

A. Organic Synthesis of SPNE:

Standard and conventional methods exist for rapid and accurate synthesis of long peptides on solid-phase supports. Solution-phase synthesis is usually feasible only for selected smaller peptides.

Synthesis on solid-phase supports, or solid-phase synthesis, is most conveniently performed on an automated peptide synthesizer according to e.g., Kent, S. et al., "Modern Methods for the Chemical Synthesis of Biologically Active Peptides," in Alitalo, K. et al., (eds.). Synthetic Peptides in Biology and Medicine, Elsevier 1985, pp. 29-57. Manual solid-phase synthesis may be employed instead, by following the classical Merrifield techniques, as described, for example, in Merrifield, R.B. J. Am. Chem. Soc. 85, 2149 (1963), or known improvements thereof. Solid-phase peptide synthesis may also be performed by the Fmoc method, which employs very dilute base to remove the Fmoc protecting group. Segment synthesis-condensation is a further variant of organic synthesis of peptides as within the scope of the techniques of the present invention.

In organic synthesis of peptides, protected amino acids are condensed to form amide or peptide bonds with the N-terminus of a growing peptide. Condensation is usually performed with the carbodiimide method by reagents such as dicyclohexyl-carbodiimide, or N-ethyl, N1-(γ-dimethylamino-propyl) carbodiimide. Other methods of forming the amide or peptide bond include, but are not limited to, synthetic routes via an acid chloride, azide, mixed anhydride or activated ester. Common solid-phase supports include polystyrene or polyamide resins.

The selection of protecting groups of amino acid side chains is, in part, dictated by particular coupling conditions, in part by the amino acid and peptide components involved in the reaction. Such amino-protecting groups ordinarily employed include those which are well known in the art, for example, urethane protecting substituents

such as benzyloxy-carbonyl (carbobenzoxy), p-methoxycarbobenzoxy, p-nitrocarbobenzoxy, t-butyloxycarbonyl, and the like. It is preferred to utilize t-butoxycarbonyl (BOC) for protecting the ε-amino group, in part because the BOC protecting group is readily removed by relatively mild acids such as trifluoroacetic acid (TFA), or hydrogen chloride in ethyl acetate.

The OH group of Thr and Ser may be protected by the Bzl (benzyl) group and the ε-amino group of Lys may be protected by the isopropoxycarbonyl (IPOC) group or the 2-chlorobenzyloxycarbonyl (2-Cl-CBZ) group. Treatment with hydrogen fluoride or catalytic hydrogenation are typically employed for removal of IPOC or 2-Cl-CBZ.

For preparing cocktails of closely related peptides, see, e.g., Houghton, R.A., Proc. Natl. Acad. Sci. USA 82, 5131 (1985).

B. Expression of SPNE in a Recombinant Expression System

It is now a relatively straightforward technology to prepare cells expressing a foreign gene. Such cells act as hosts and include E. coli, B. subtilis, yeasts, fungi, plant cells or animal cells. Expression vectors for many of these host cells have been isolated and characterized, and are used as starting materials in the construction, through conventional recombinant DNA techniques, of vectors having a foreign DNA insert of interest. Any DNA is foreign if it does not naturally derive from the host cells used to express the DNA insert. The foreign DNA insert may be expressed on extra-chromosomal plasmids or after integration in whole or in part in the host cell chromosome(s), or may actually exist in the host cell as a combination of more than one molecular form. The choice of host cell and expression vector for the expression of a desired foreign DNA largely depends on availability of the host cell and how fastidious it is, whether the host cell will support the replication of the expression vector, and other factors readily appreciated by those of ordinary skill in the art.

The technology for recombinant procaryotic expression systems is now old and conventional. The typical host cell is E. coli.

The technology is illustrated by treatises such as Wu, R (ed) Meth. Enzymol. 68 (1979) and Maniatis, T. et. al., Molecular Cloning: A Laboratory Manual Cold Spring Harbor 1982.

The foreign DNA insert of interest comprises any DNA sequence coding for a SPNE (or fragment thereof of at least 5 amino acids in length) of the present invention, including any synthetic sequence with this coding capacity or any such cloned sequence or combination thereof. For example, SPNE peptides coded and expressed by an entirely recombinant DNA sequence is encompassed by this invention.

Vectors useful for constructing eukaryotic expression systems for the production of recombinant SPNE comprise the DNA sequence for SPNE, fragment or variant thereof, operatively linked thereto with appropriate transcriptional activation DNA sequences, such as a promoter and/or operator. Other typical features may include appropriate ribosome binding sites, termination codons, enhancers, terminators, or replicon elements. These additional features can be inserted into the vector at the appropriate site or sites by conventional splicing techniques such as restriction endonuclease digestion and ligation.

Yeast expression systems, which are one variety of recombinant eukaryotic expression systems, generally employ Saccharomyces cerevisiae as the species of choice for expressing recombinant proteins. S. cerevisiae and similar yeasts possess well known promoters useful in the construction of yeast expression systems, including but not limited to GAP491, GAL10, ADH2, and alpha mating factor.

Yeast vectors useful for constructing recombinant yeast expression systems for expressing SPNE include, but are not limited to, shuttle vectors, cosmids, chimeric plasmids, and those having sequences derived from 2-micron circle plasmids.

Insertion of the appropriate DNA sequence coding for SPNE, fragment or variant thereof, into these vectors will, in principle, result in a useful recombinant yeast expression system for SPNE where

the modified vector is inserted into the appropriate host cell, by transformation or other means.

Recombinant mammalian expression systems are another means of producing the recombinant SPNE for the conjugates of this invention. In general, a host mammalian cell can be any cell that has been efficiently cloned in cell culture. Host mammalian cells useful for the purposes of constructing a recombinant mammalian expression system include, but are not limited to, Vero cells, NIH3T3, GH3, COS, murine C127 or mouse L cells. Mammalian expression vectors can be based on virus vectors, plasmid vectors which may have SV40, BPV or other viral replicons, or vectors without a replicon for animal cells. Detailed discussions on mammalian expression vectors can be found in the treatises of Glover, D.M. (ed.) "DNA Cloning: A Practical Approach," IRL 1985, Vols. I and II.

Recombinant SPNE may possess additional and desirable structural modifications not shared with the same organically synthesized peptide, such as adenylation, carboxylation, glycosylation, hydroxylation, methylation, phosphorylation or myristoylation. These added features may be chosen or preferred as the case may be, by the appropriate choice of recombinant expression system. On the other hand, recombinant SPNE may have its sequence extended by the principles and practice of organic synthesis of section A above.

CONJUGATION OF SPNE AND OMPC TO FORM A COVALENT LINKAGE(S) YIELDING CONJUGATE OR COCONJUGATE

Antigenic conjugates of SPNE and OMPC are useful for vaccination against AIDS or ARC. Such conjugates have at least one covalent linkage between the antigen SPNE and OMPC, and typically have more than one SPNE molecule covalently bound to each OMPC molecule.

SPNE and OMPC are prepared separately, then linked by non-specific cross-linking agents, monogeneric spacers or bigeneric spacers. Methods for non-specific cross-linking include, but are not limited to, reaction with glutaraldehyde; reaction with N-ethyl-N'-(3-

dimethylaminopropyl) carbodiimide, with or without admixture of a succinylated carrier; periodate oxidation of glycosylated substituents followed by coupling to free amino groups of a protein carrier in the presence of sodium borohydride or sodium cyanoborohydride; diazotization of aromatic amino groups followed by coupling on tyrosine side chain residues of the protein; reaction with isocyanates; or reaction of mixed anhydrides. See, generally, Briand, J.P. et al. J. Imm. Meth. 78, 59 (1985). These methods of non-specifically cross-linking are conventional and well-known in the typical practice of preparing conjugates for immunological purposes.

In another embodiment of the invention, conjugates formed with a monogeneric spacer are prepared. These spacers are bifunctional and require functionalization of only one of the partners of the reaction pair to be conjugated before conjugation takes place.

By way of illustration rather than limitation, an example of a monogeneric spacer involves coupling the polypeptide SPNE to one end of the bifunctional molecule adipic acid dihydrazide in the presence of carbodiimide. A diacylated hydrazine presumably forms with pendant glutamic or aspartic carboxyl groups of SPNE. Conjugation then is performed by a second coupling reaction with carrier protein in the presence of carbodiimide. For similar procedures, see for example, Schneerson, R. et al., J. Exp. Med. 152, 361 (1980). Another example of a monogeneric spacer is described in Fujii, N. et al. Int. J. Peptide Protein Res. 26, 121 (1985).

In another embodiment of the invention, conjugates of SPNE and OMPC are formed with a bigeneric spacer. These spacers are formed after each partner of the reaction pair to be conjugated, e.g., SPNE and OMPC, is functionalized with a bifunctional spacer. Conjugation occurs when each functionalized partner is reacted with its opposite partner to form a stable covalent bond or bonds. See, for example, Marburg, S. et al., J. Am. Chem. Soc. 108, 5282-5287 (1986) and Marburg, S. et al., U.S. Patent 4,695,624, issued 22 September 1987. Bigeneric spacers are preferred for preparing conjugates in

human vaccines since the conjugation reaction is well characterized and easily controlled.

In another embodiment of this invention, coconjugates are formed of SPNE and OMPC, comprising conjugates of SPNE and OMPC wherein OMPC is also covalently modified with a low molecular weight moiety (hereinafter a-) having an anionic or polyanionic character at physiological pH. The term a- is typically one to five residues of an anionic form of carboxylic, sulfonic, propionic or phosphonic acid. Such coconjugates are suitable for raising an anti-SPNE response, since the anions enhance solubility of conjugates in aqueous solutions. Their synthesis, detailed description and other advantages are described in EPO467700 of Leanza, W.J. et al.

Typical and conventional immunological practice provides for the ready and easy synthesis of antigenic conjugates within the scope of the present invention, including the conjugation of OMPC with virtually any desired degree of substitution of virtually any peptide of the Sequence Listing. Heterogeneous products of the conjugation reaction are easily separable if needed by a variety of suitable column chromatography techniques.

RECOMBINANT FUSION POLYPEPTIDES (RFPS)

For ease in evaluating SPNE as immunogens, applicants have contructed recombinent shuttle vectors coding for RFPs of novel SPNE and selected peptides or fragments thereof, such as pIII (with or without a polyhistidine tail), Hep B core, Hep B surface antigen or protein A. The methods for contruction of fusion peptides are well known in the art. Coding sequences are prepared by ligation of other sequences, cloning, PCR, mutagenesis, organic synthesis, or combination thereof, in accordance with the principles and practice of constructing DNA sequences.

For the particular RFPs of this invention, DNA sequences coding for a selected SPNE are ligated in frame to DNA sequences coding for pIII, Hep B core or protein A. The resulting DNA fragment is expressed in any one of a wide variety of readily available

recombinant expression systems, e.g. E. coli BL21 (DE3), as also discussed in the Examples and in the section on expression of SPNE in a recombinant expression system, above.

In the alternative, the fusion peptides can be made by synthetic organic means, although this method is limited by feasibility and by practicality to smaller fusion peptides. See also the section on organic synthesis of SPNE, above.

VACCINE FORMULATION

The form of the immunogen within the vaccine takes various molecular configurations. A single molecular species of the antigenic conjugate (SPNE)n-OMPC will often suffice as a useful and suitable antigen for the prevention or treatment of AIDS or ARC. Other antigens in the form of cocktails are also advantageous, and consist of a mixture of conjugates that differ by, for example, the degree of substitution (n) or the amino acid sequence of SPNE or both.

An immunological vector or adjuvant may be added as an immunological vehicle according to conventional immunological testing or practice.

The conjugates of this invention when used as a vaccine, are to be administered in immunologically effective amounts. Dosages of between 1 μg and 500 μg of conjugate, and preferably between 50 μg and 300 μg of conjugate are to be administered to a mammal to induce anti-peptide, anti-HIV, or HIV-neutralizing immune responses. About two weeks after the initial administration, a booster dose may be administered, and then again whenever serum antibody titers diminish. The conjugate should be given intramuscularly at a concentration of between 10 μg/ml and 1 mg/ml, and preferably between 50 and 500 μg/ml, in a volume sufficient to make up the total required for immunological efficacy.

Adjuvants may or may not be added during the preparation of the vaccines of this invention. Alum is the typical and preferred adjuvant in human vaccines, especially in the form of a thixotropic, viscous, and homogeneous aluminum hydroxide gel. For example, one

embodiment of the present invention is the prophylactic vaccination of patients with a suspension of alum adjuvant as vehicle and a cocktail of $(SPNE)_n$-OMPC as the selected set of immunogens or antigens.

OTHER UTILITIES

The SPNEs and their immunological conjugates in this invention are also useful in screening blood products for the presence of HIV antigen or HIV-specific antibody. Thus, $(SPNE)_n$~OMPC or SPNE can be readily employed in a variety of immunological assays of the type well known to the skilled artisan, e.g., radioimmunoassay, competitive radioimmunoassay, enzyme-linked immunoassay, and the like. For an extensive discussion of these types of utilities, see, e.g. U.S. 5,075,211.

METHOD FOR SCREENING PHAGE EPITOPE LIBRARIES

Phage epitope libraries are unusually versatile vehicles for identifying new antigens or ligands. Typically, the phage has inserted into its genome a small, randomly generated DNA sequence, e.g. 45 base pairs, which will generate exposed oligo-peptide surfaces in the mature phage. Mixing a library of such mature phage with a screening antibody of desired specificity, followed by separation of bound from unbound phage, allows the opportunity to clone and sequence the bound phage. A conventional example of a phage epitope library is the filamentous phage fd and its gene III coding for minor coat protein pIII. See, e.g., Parmley, S. F. et al. Gene 73, 305 (1988) and Scott, J. K. et al. Science 249, 386 (1990), which set forth extensive discussion and detail on construction of these libraries.

Applicants have developed a new method for screening phage epitope libraries. The screening method involves selection of epitopes by binding to a solid-phase supported antibody, optionally followed by identification of desired clones with antibody lifts. The screening method is useful for virtually any antibody, i.e. polyclonal or monoclonal or collection of monoclonals thereto. Any antigen can be

screened. The screening method is illustrated by HIV antigens screened with an HIV-specific broadly neutralizing antibody.

The present screening method avoids the typical prior art problem of biotin-avidin complexes. Although, biotin-avidin complex formation has an unusually high binding constant, it produces false positives, is time-consuming, and requires tampering with the antibody by covalent conjugation. Applicants avoid all of these problems by adsorbing the antibody onto a solid-phase support. With a particular series of mixing and washing steps, applicants demonstrate a practical method of screening phage libraries.

Screening in the present invention is broken down into two separate methods. The first method involves selection of desired phage epitopes with a solid-phase supported antibody of any desired specificity. The second method, which is optional, relates to identification of desired phage epitopes by antibody lifts.

A. Selection

Selection of desired phage epitopes in a phage epitope library is performed as follows. An essentially pure preparation of monospecific antibody is adsorbed or otherwise attached to a solid-phase support, hereinafter also referred to as solid-phase supported Ab. The most preferred embodiment is monoclonal antibody adsorbed to polystyrene beads large enough to be picked up with tweezers, e.g., with a diameter of 0.25 inch. Such large beads contribute to the ease of subsequent washing steps. Other embodiments include any solid-phase adsorbent for antibody, or any plastic, or glass bead or polysaccharide gel, e.g. Sepharose. Polysaccharide gels are typically covalently conjugated to the purified antibody by, e.g., cyanogen bromide activation.

Incubation of the solid-phase supported Ab with BSA, milk solids or other reagent for blocking non-specific interactions is preferable before selection. The presence of low levels of a mild or nonionic detergent is desirable, e.g., 0.5%(v/v) of one or more in the polyoxyethylene (20) sorbitan monoleate series (TWEEN), or

octylglucopyranoside or Nonidet NP-40. It is apparent to the skilled how to adjust the conditions for coating with such blocking agents.

An appropriate density of antibody should be determined by titration. Applicants have successfully performed selection with a density of about 0.1 μg antibody/cm^2 on polystyrene beads (d = 0.25 inch). This falls within a preferred density range of between about 1mg Ab/cm^2 and about 0.1 μg Ab/cm^2. Densities of a still lower range select high affinity epitopes because of the reduced incidence of multivalent binding by the antibody to the multiple copies of the epitope on the phage tip. It is apparent to the skilled artisan how to determine the most suitable density for an antibody preparation, by monitoring the bound phage population. As a general rule, a manageable complexity of bound and eluted phage ranges from about $5x10^3$ to about 10^5 phage.

Throughout the selection method described below, a wide variation in incubation times, washing times, temperature and pH is covered. It is apparent to the skilled artisan that, given a particular incubation or washing step, a suitable set of variant reaction conditions can be readily ascertained. Applicants have discovered that temperature and pH are critical in the stringent selection of high affinity epitopes, e.g., temperatures exceeding about 70°C at neutral pH, or exceeding about 38°C at pH 4.0, are lethal to the phage. Aside from the critical parameters of temperature and pH, the typical buffer is isotonic to saline, and may contain a non-specific blocking agent such as bovine serum albumin (BSA) or milk solids, as well as low levels of a nonionic detergent. For example, TTBS (50mM Tris pH 7.5, 150 mM NaCl, 0.5% (v/v) TWEEN-20) in 1mg/ml BSA is typical.

Solid-phase supported antibody is first incubated with the epitope phage library to effect binding of the phage epitopes to the antibody. It is preferred to use enough phage to vastly exceed the library complexity, e.g., 10^{11} phage which is 1000 fold more than its complexity of 10^8. Incubation between about 4°C and about 65°C, for at least 10 minutes is performed. Applicants typically incubate overnight at 4°C. Alternatively, a one hour incubation at 37°C will select epitopes binding at a fast "on" or forward rate. Incubation

conditions are subject to a wide range of variations, as also discussed above, but a neutral buffer containing a non-specific blocking agent is preferred, e.g., TTBS, 1 mg/ml BSA.

Washing of the mixture of phage epitope library and solid-phase supported antibody to remove unbound phage is carried out in a variety of conditions, depending on the desired stringency. The higher the desired stringency, the higher the temperature conditions of washing, up to 70°C in some conditions.

For high stringency selection, washing of the mixture is carried out by washing 3 to 20 times in buffer at neutral pH at 65°C without blocking agent (hereinafter the 65°C wash). Low-affinity phage epitopes are then eluted by washing one or more times by brief (2-5 minutes) immersion in a mildly acidic buffer without blocking agent (about pH 4.0, between 5.0 and 3.0) at ambient temperature or between about 4°C and 37°C (the pH 4.0 wash). The pH 4.0 wash is optional in high stringency selection, but it cannot be completely combined with the 65°C wash. For example, the phage die in pH 4.0 buffer at 65°C.

High stringency selection may be enhanced by lowering the antibody density on the bead or other solid-phase support. In this case, lowering the probability that a given phage will bind more than one antibody molecule selects for higher affinity epitopes. It will be apparent to those skilled in the art how to test density variations within the aforementioned ranges.

Lower stringency selection is performed instead by washing 3 to 20 times at neutral pH at about room temperature. A pH 4.0 wash may optionally follow.

Elution of high affinity epitopes is the next required step (hereinafter the pH 2.0 elution) for both high and low stringency selection. Phage bound to solid-phase supported antibody are incubated briefly (1-15 minutes) in a low pH buffer in about 0.1-10 mg/ml BSA or other non-specific binder. The buffer pH can vary from about 2.3 to about 1.0, but 2.2 is preferred. Temperature conditions range from about 37°C to 4°C, room temperature being desirable. Preferred

buffered conditions are 0.1N glycine•HCl pH 2.2, 1 mg/ml BSA at room temperature.

After the pH 2.0 elution, the eluted solution containing phage is neutralized by standard and well-known techniques. The eluted phage are grown in infectable E. coli, e.g. tet^+ phage are grown in tet^- E.coli on media containing tetracycline.

Thus concludes one cycle of selection, either at high stringency or low stringency. Repetition of the cycle after growth of the phage is often found advantageous, as it lowers the complexity of eluted phage to manageable magnitudes (less than about 10^5). Repeating the cycle 2-10 times, preferably 3-5 times, is found most practical. It will be apparent to those skilled in the art that indicated variations are readily performed and evaluated, such as switching from high stringency to low stringency on the second cycle of selection, or changing the buffer or its pH.

B. Identification With Antibody Lifts

After selection of epitopes bound to phage, it is advantageous to identify with antibody lifts those clones with desired epitopes. The principle is to overlay culture plates of cells infected with selected phage epitopes, remove the overlay, block the overlay, incubate the blocked overlay with desired antibody, label the bound antibody, and locate on the original culture plate those colonies that bind the antibody. Versions of this overlay technique that differ from the present method exist in the literature. Methods known in the art are typically adopted for use with plaque formers, unlike the present invention. See, e.g., Young, R.A. et al., Proc Natl. Acad Sci 80, 1194 (1983); Ausubel, F.M. et al. (eds.), "Screening Recombinant DNA Libraries," in Current Protocols in Molecular Biology, Chapter 6, Greene 1989; and Davis, L.G. et al., Basic Methods in Molecular Biology, pp. 214-215, Elsevier 1986.

Plates having epitope phage-infected colonies are grown to the extent that the colonies are sufficiently large, i.e., between about 1mm and about 4mm in diameter, yielding mature plates.

Mature plates are overlaid with a disk that binds proteins. The disc is typically nitrocellulose, but it may also be IMMOBILON P, cellulose acetate and the like. The disk is immediately removed and subjected to further treatment.

Blocking the overlay or disk is first performed to eliminate or substantially reduce the background of non-specific interactions. Useful blocking agents include BSA, milk solids and similar proteinaceous preparations. The disks are soaked for at least 2 hours in buffer, containing between about 0.1% (v/v) and about 1.0% (v/v) neutral detergent and at least 1% blocking agent. One preferred embodiment for this blocking step is soaking for 4 hours each disk in TTBS, 10% evaporated milk, at room temperature. A preferred range is incubation for at least 2 hours, in a buffer near neutrality (5.0-8.0) containing 0.1% (v/v) - 1.0% (v/v) neutral detergent, in about 1% to about 20% blocking agent, within a temperature range of about 4°C to about 80°C.

Washing the blocked disks to remove excess blocking agent follows, and is carried out in a buffer lacking the blocking agent. One preferred embodiment for this washing step is soaking each disk two or three times in TTBS, pH 7.3-7.5, at room temperature. A preferred range of conditions is soaking for at least 10 minutes, in a buffer with a pH that does not destroy antibody (5.0-8.0), containing 0.1% (v/v) to 1.0% (v/v) neutral detergent, within a temperature range of about 4°C to about 80°C.

Contacting the disk with screening antibody follows. One preferred embodiment is incubating the washed disks overnight at 4°C with gentle rocking, in TTBS, 1% evaporated milk, 0.5 to 1.0 µg/ml antibody. A preferred range of conditions is incubating the disks for at least 4 hours, within a temperature range of between about 4°C and about 65°C, in buffer near neutrality containing about 0.1% (v/v) to about 1.0% (v/v) neutral detergent, in 0.1% to 5% blocking agent, and 0.1 to 5 µg/ul antibody.

A second series of washes are performed, here to remove excess or unbound antibody. One preferred embodiment is soaking

each disk four times in TTBS for 20 minutes at room temperature. Preferred ranges of conditions are at least 2 soaks in buffer without blocking agents at a pH near neutrality (6.0-8.0), for 5 minutes to 1 hour, between about 10°C and 45°C.

The resulting washed disks having bound antibody are treated with a labeled second-stage reagent to determine the location of the bound antibody and the corresponding epitope clone. Any labeled or tagged second-stage reagent useful for binding the bound antibody can in principle be incorporated into the procedure for the purposes of identifying the clones having epitopes bound by antibody. One preferred embodiment is soaking the washed disks having bound antibody in TTBS, 1% milk, ^{125}I-protein A (0.5 to 1μ curie/ml) for 1.5 to 3 hours. Preferred ranges of conditions are incubating the disks for at least 1 hour, within a temperature range of between about 4°C to about 65°C, in buffer near neutrality containing about 0.1% (v/v) to about 1.0% (v/v) neutral detergent, in about 0.1% to about 5% blocking agent and detectable quantities of labeled protein A. Another preferred second-stage reagent is labeled protein G, e.g., ^{125}I-protein G. Other appropriate second-stage reagents include, but are not limited to, double antibody, such as ^{125}I-labeled mouse anti-human IgG, or mouse anti-human IgG tagged with beta-galactosidase or peroxidase. Substantial purity of labeled second-stage reagent is desirable.

The disks having bound labeled antibody are now soaked or washed to remove unbound label. One preferred embodiment is soaking 20 minutes four times in TTBS. The location of the labeled, bound antibody on the disks is determined by conventional procedures appropriate for the labeled second-stage reagent.

X-ray film is used for ^{125}I. Chromogenic substrates are useful in a variety of enzyme-antibody detection kits.

Once the location of the bound antibody is determined, e.g., a pattern of dark spots on developed X-ray film, one identifies the appropriate colonies on the original mature plate, since regrown as needed. Subsequent replating, growth, and sequencing gives a particular selected principal neutralizing epitope (SPNE).

COMBINATION THERAPY

The vaccines of this invention may be effectively administered, whether at periods of pre-exposure and/or post-exposure, in combination with effective amounts of the AIDS antivirals, immunomodulators, anti-infectives, or vaccines of the following Table.

TABLE I

ANTI-VIRALS

Drug Name	Manufacturer	Indication
AL-721	Ethigen (Los Angeles, CA)	ARC, PGL HIV positive, AIDS
Recombinant Human Interferon Beta	Triton Biosciences (Almeda, CA)	AIDS, Kaposi's sarcoma, ARC
Acemannan	Carrington Labs (Irving, TX)	ARC (See also immunomodulators)
Cytovene Ganciclovir	Syntex (Palo Alto, CA)	sight threateining CMV peripheral CMV retinitis
d4T Didehydrodeoxythymidine	Bristol-Myers (New York, NY)	AIDS, ARC
ddI Dideoxyinosine	Bristol-Myers (New York, NY)	AIDS, ARC

Drug Name	Manufacturer	Indication
EL10	Elan Corp, PLC (Gainesville, GA)	HIV infection (See also immuno-modulators)
Foscarnet Trisodium Phosphonoformate	Astra Pharm. Products, Inc. (Westborough, MA)	CMV retinitis, HIV infection, other CMV infections
L-697,661	Merck (Rahway, NJ)	AIDS, ARC, HIV positive asymptomatic, or in combination with AZT, ddC or ddI. Inhibitor of HIV RT
L-735,524	Merck (Rahway, NJ)	AIDS, ARC, HIV positive asymptomatic, or in combination with AZT, ddC or ddI. Inhibitor of HIV Protease, not HIV RT
Dideoxycytidine; ddC	Hoffman-La Roche (Nutley, NJ)	AIDS, ARC

Drug Name	Manufacturer	Indication
Novapren	Novaferon Labs, Inc. (Akron, OH) Diapren, Inc. (Roseville, MN, marketer)	HIV inhibitor
Peptide T Octapeptide Sequence	Peninsula Labs (Belmont, CA)	AIDS
Retrovir Zidovudine; AZT	Burroughs Wellcome (Rsch. Triangle Park, NC)	AIDS, adv, ARC pediatric AIDS, Kaposi's sarcoma, asymptomatic HIV infection, less severe HIV disease, neurological involvement, in combination w/ other therapies, post-exposure prophylaxis in health care workers
Rifabutin Ansamycin LM 427	Adria Laboratories (Dublin. OH) Erbamont (Stamford, CT)	ARC
Dextran Sulfate	Ueno Fine Chem. Ind. Ltd. (Osaka, Japan)	AIDS, ARC, HIV positive asymptomatic

Drug Name	Manufacturer	Indication
Virazole Ribavirin	Viratek/ICN (Costa Mesa, CA)	asymptomatic HIV positive, LAS, ARC
Alpha Interferon	Burroughs Wellcome (Rsch. Triangle Park, NC)	Kaposi's sarcoma, HIV in combination w/Retrovir
	IMMUNO-MODULATORS	
Antibody which neutralizes pH labile alpha aberrant Interferon in an immunoadsorption column	Advanced Biotherapy Concepts (Rockville, MD)	AIDS, ARC
AS-101	Wyeth-Ayerst Labs. (Philadelphia, PA)	AIDS
Bropirimine	Upjohn (Kalamazoo, MI)	advanced AIDS
Acemannan	Carrington Labs, Inc. (Irving, TX)	AIDS, ARC (See also antivirals)
CL246,738	American Cyanamid (Pearl River, NY) Lederle Labs (Wayne, NJ)	AIDS, Kaposi's sarcoma
EL10	Elan Corp, PLC (Gainesville, GA)	HIV infection (See also antivirals)

Drug Name	Manufacturer	Indication
Gamma Interferon	Genentech (S. San Francisco, CA)	ARC, in combination w/TNF (tumor necrosis factor)
Granulocyte Macrophage Colony Stimulating Factor	Genetics Institute (Cambridge, MA) Sandoz (East Hanover, NJ)	AIDS
Granulocyte Macrophage Colony Stimulating Factor	Hoeschst-Roussel (Somerville, NJ) Immunex (Seattle, WA)	AIDS
Granulocyte Macrophage Colony Stimulating Factor	Schering-Plough (Madison, NJ)	AIDS AIDS, in combination w/Retrovir
HIV Core Particle Immunostimulant	Rorer (Ft. Washington, PA)	seropositive HIV
IL-2 Interleukin-2	Cetus (Emerycille, CA)	AIDS, in combaintion w/Retrovir
IL-2 Interleukin-2	Hoffman-La Roche (Nutley, NJ)	AIDS, ARC, HIV, in combination w/Retrovir
Immune Globulin Intravenous (human)	Cutter Biological (Berkeley, CA)	pediatric AIDS, in combination w/Retrovir

Drug Name	Manufacturer	Indication
IMREG-1	Imreg (New Orleans, LA)	AIDS, Kaposi's sarcoma, ARC, PGL
IMREG-2	Imreg (New Orleans, LA)	AIDS, Kaposi's sarcoma, ARC, PGL
Imuthiol Diethyl Dithio Carbamate	Merieux Institute (Miami, FL)	AIDS, ARC
INTRON A Alpha-2 Interferon	Schering Plough (Madison, NJ)	Kaposi's sarcoma w/Retrovir: AIDS
Methionine-Enkephalin	TNI Pharmaceutical (Chicago, IL)	AIDS, ARC
MTP-PE Muramyl-Tripeptide	Ciba-Geigy Corp. (Summit, NJ)	Kaposi's sarcoma
Granulocyte Colony Stimulating Factor	Amgen (Thousand Oaks, CA)	AIDS, in combination w/Retrovir
rCD4 Recombinant Soluble Human CD4	Genentech (S. San Francisco, CA)	AIDS, ARC
Recombinant Soluble Human CD4	Biogen (Cambridge, MA)	AIDS, ARC

Drug Name	Manufacturer	Indication
Roferon-A Interferon Alfa 2a	Hoffman-La Roche (Nutley, NJ)	Kaposi's sarcoma AIDS, ARC, in combination w/Retrovir
SK&F106528 Soluble T4	Smith, Kline & French Laboratories (Philadelphia, PA)	HIV infection
Thymopentin	Immunobiology Research Institute (Annandale, NJ)	HIV infection
Tumor Necrosis Factor; TNF	Genentech (S. San Francisco, CA)	ARC, in combination w/gamma Interferon
	ANTI-INFECTIVES	
Clindamycin with Primaquine	Upjohn (Kalamazoo, MI)	PCP
Diflucan Fluconazole	Pfizer (New York, NY)	cryptococcal meningitis, candidiasis
Pastille Nystatin Pastille	Squibb Corp. (Princeton, NJ)	prevention of oral candidiasis
Ornidyl Eflornithine	Merrell Dow (Cincinnati, OH)	PCP

Drug Name	Manufacturer	Indication
Pentamidine Isethionate (IM & IV)	LyphoMed (Rosemont, IL)	PCP treatment
Piritrexim	Burroughs Wellcome (Rsch. Triangle Park, NC)	PCP treatment
Pentamidine isethionate for inhalation	Fisons Corporation (Bedford, MA)	PCP prophylaxis
Spiramycin	Phone-Poulenc Pharmaceuticals (Princeton, NJ)	cryptosporidial diarrhea
Intraconazole-R51211	Janssen Pharm. (Piscataway, NJ)	histoplasmosis; cryptococcal meningitis
Trimetrexate	Warner-Lambert	PCP
	OTHER	
Recombinant Human Erythropoietin	Ortho Pharm. Corp. (Raritan, NJ)	severe anemia assoc. and Retrovir therapy
Megestrol Acetate	Bristol-Myers (New York, NY)	treatment of anorexia assoc. w/AIDS

It will be understood that the scope of combinations of the antigenic conjugates of this invention with AIDS antivirals, immunomodulators, anti-infectives or vaccines is not limited to the list in the above Table, but includes in principle any combination with any pharmaceutical composition useful for the treatment of AIDS. The antigenic conjugates as AIDS or HIV vaccines of this invention include vaccines to be used pre- or post-exposure to prevent or treat HIV infection or disease, and are capable of producing an immune response specific for the immunogen.

The compound L-697,661 is 3-([4,7-dichloro-1,3-benzoxazol-2-yl)methyl]amino)-5-ethyl-6-methyl-pyridin-2(1H)-one or pharmaceutically acceptable salt thereof. The compound L-735,524 is N-(2(R)-hydroxy-1(S)-indanyl)-2(R)-phenylmethyl-4-(S)-hydroxy-5-(1-(4-(3-pyridyl-methyl)-2(S)-N'-(t-butylcarboxamido)-piperazinyl))-pentaneamide, or pharmaceutically acceptable salt thereof.

SOURCE OF MONOCLONAL ANTIBODY

The 2F5 antibody is purchased from Waldheim Pharmazeutika, Catalog Number IAM 41-2F5.

EXAMPLE 1

Library Construction

A. Random Library

A phage library containing random 15 amino acid epitopes was constructed by the methods of Scott, J.K. et al. Science 249, 386 (1990). In this protocol, synthetic 110 bp BglI fragments were prepared containing the degenerate coding sequence (NNK)15, wherein N stands for an equal mixture of G, A, T and C, and K stands for an equal mixture of G and T. The library was constructed by ligating the synthetic 110 bp BglI fragments in phage fUSE5 and transfecting E. coli cells with the ligation product by electroporation.

The resulting phage oligopeptide epitope library (also known as Library ALPHA) had a complexity of approximately 50 x 10^6

different epitopes. Flanking nucleotide sequences were added to enhance folding of the amino acid sequence.

B. Semi Random Libraries

The following semi-random libraries were constructed in the same manner as Example 1A:

TABLE OF LIBRARIES

LIBRARY	Peptide Sequence	Complexity	SEQ.ID.
ALPHA	ADGAXXXXXXXXXXXXXXXGAAGA	$50x10^6$	26
BETA	ADGAXXXXXXXXXGPXRXXGAAGA	$92X10^6$	27
GAMMA	ADGALLXXXXXGPXRXXXXXLLGAAGA	$66X10^6$	28
DELTA	ADGACXXXXXGPXRXXXXXCGAAGA	$45X10^6$	29
EPSILON	ADGACXXXXXXXXXXXXXXCGAAGA	$200X10^6$	30

X is any amino acid

Library BETA consists of random polypeptide sequences around the V3 loop region consensus sequence; library GAMMA adds terminal leucines for potential loop formation; library DELTA instead adds a terminal cysteine on each end for potential loop formation; library EPSILON is a control of any sequence with a cysteine loop.

The monoclonal antibody 25F was screened against the ALPHA Library, to give the amino acid sequences of the Sequence Listing.

EXAMPLE 2

Bead Coating Procedure

Polystyrene beads (d = 0.25 inch) were coated with between 1 and 10 µg of antibody per ml in 50 mM Na_2CO_3, pH 9.6, 0.02% sodium azide. (Note that any solid phase adsorbent should work). Beads were incubated in the antibody solution at 4°C overnight.

The next day the coated beads were washed 3x with phosphate buffered saline and 1x with water. After washing, the antibody-coated beads were air dried and stored frozen at -20°C until needed. Before use, the antibody-coated beads were coated with 10 mg/ml BSA (to block free sites on the plastic) in TTBS (50 mM Tris pH 7.5, 150 mM NaCl, 0.5% (v/v) Tween 20) for 4 or more hours. Each batch of beads was checked for antibody activity by its ability to bind ^{125}I protein A, before being used in a phage selection screen.

EXAMPLE 3

Stringent Phage Selection with Antibody-Coated Beads

A. First Method-Low Stringency

The random epitope phage library ALPHA was incubated at 4°C overnight with gentle rocking, with antibody-coated beads in TTBS, 1 mg/ml BSA. Typically, a total volume of 1cc containing about 10^{11} total phage was used. The next day the bead, containing bound phage, was washed 10 to 12 times in TTBS, in a volume of 10cc per wash, at room temperature, with a gentle rocking motion, for 10 minutes per wash. The liquid was carefully drained off the bead between each wash. After the last wash the bound phage were eluted off the bead by incubating for 5 minutes at room temperature in a minimal volume (typically 200 µl) of 0.1N HCl, adjusted to pH 2.2 with glycine, 1mg/ml BSA. The solution with the eluted phage was neutralized by adding 12 µl of 2M Tris, pH unadjusted, per 200 µl phage solution. The eluted phage were then used to infect E. coli K91K cells. Infected cells were plated onto LB agar plates containing 40 mg/ml tetracycline. Since the phage carry a tetracycline resistance marker, only infected cells grow on the plates. Typically, one bead selected between 5000 and 100,000 independent phage.

B. Second Method-High Stringency

The random epitope library or semi-random library was incubated at 4°C overnight with gentle rocking, with antibody-coated beads in TTBS, 1 mg/ml BSA. Typically, a total volume of 1cc containing on the order of 10^{11} total phage was used, corresponding to the complexity of the library x 1000. The next day the bead containing the bound phage was washed 10 times in TTBS, in a volume of 10cc per wash, at 65°C, with gentle rocking, for 10 minutes per wash. Note that 65°C in TTBS does not destroy phage. There followed one wash at room temperature in TTBS pH 4.0. The liquid was carefully drained off the bead between each wash. Next, the bound phage were eluted off the bead by incubating for 5 minutes at room temperature in 200 µl of 0.1N HCl, adjusted to pH 2.2 with glycine, 1 mg/ml BSA. The phage solution was neutralized by adding 12 ml of 2M Tris, pH unadjusted. The eluted phage were then used to infect E. coli K91K cells. Infected cells were grown in 1 x Luria broth containing 40 µg/ml tetracycline (250 cc) and incubated with shaking for 48 hours at 37°C. Phage were harvested and precipitated twice with PEG (polyethylene glycol). The precipitated phage were then titered and approximately 10^{10} of the first round selected phage were again incubated with an antibody coated bead, washed as described above, regrown and harvested. Three cycles of selection and growth were performed. E. coli infected with phage were plated as clonal isolates.

EXAMPLE 4

Screening of Selected Phage with Antibody Lifts

After 1 or more rounds of selection according to Example 3, the infected E. coli colonies were screened for the ability to bind antibody (using the same antibody as used to select the phage). This was done by growing the plates until the colonies reached a diameter of one to four mm, placing nitrocellulose disks onto the plates, lifting the disks and placing them in a solution of 10% evaporated milk, TTBS for 4 or more hours. After lifting, the plate containing the infected colonies

were regrown for several hours at 37°C and placed at 4°C until needed. The nitrocellulose disks, at the end of 4 or more hours in the solution of 10% evaporated milk and TTBS, were washed 2-3x in TTBS and placed in TTBS and 1% milk and 0.5 to 1 µg/ml antibody solution. They were then incubated at 4°C overnight with gentle rocking. After incubation in the antibody solution, the disks were washed 4x in 100cc TTBS for 20 minutes with gentle rocking. They were then incubated in TTBS and 1% milk and I^{125} protein A (.5 to 1 µ curie/ml) for 1-1/2 to 3 hours. The disks were again washed 4x in 100 cc TTBS for 20 minutes. They were placed on X-ray film for 12 to 72 hours. The film was developed and colonies corresponding to dark spots were picked. If the plates were too dense to pick isolated colonies, the picked colony(ies) was replated at a lower density and the screen repeated to get clonal isolates.

EXAMPLE 5

PCR Sequencing

Phage infected E. coli K91K cells were grown overnight at 37°C in 1x Luria broth, 40 µg/ml tetracycline on a rollerdrum. The cells were pelleted and 1 µl of supernatant was used as the template in PCR reactions. The template was amplified using a 100-fold excess of one primer over the other. Template and oligonucleotide primers (Primer 1008: 5'-TCG AAA GCA AGC TGA TAA ACC G-3' SEQ ID NO:32, located 106 nucleotides upstream of random insert and Primer 1009: 5'-ACA GAC AGC CCT CAT AGT TAG CG-3' SEQ ID NO:33, located 87 nucleotides downstream from random insert) were reacted in a volume of 100 µl containing 50 mM KCl, 10 mM Tris-HCl pH 8.3, 1.5 mM $MgCl_2$, 0.01% (w/v) gelatin, 200 µM each dNTP, and 2.5 units Taq polymerase. Reactions were overlaid with mineral oil and amplified in a thermal cycler for an initial 8 minute 94°C incubation, then 30 cycles of 30 seconds at 94°C, 1 minute at 55°C and 2 minutes at 72°C followed by a 5 minute incubation at 72°C. The mineral oil was removed, 2 ml of water added to the reactions, and the sample centrifuged in a microconcentrator for 30 minutes at 1000 x g. The

retentate volume was brought to 2 ml with water and centrifuged as above. The retentate was then collected by centrifugation for 2 minutes at 500 x g. Retentate concentrations were determined by electrophoresis on a 1% agarose gel containing 0.5 µg/ml Ethidium bromide and visualization under ultraviolet light. The retentate was dried along with enough limiting primer from PCR reaction (or internal primer 1059-5'GTA AAT GAA TTT TCT GTA TGA GG 3' SEQ ID NO:31 located 27 nucleotides downstream from insert) to give a 5:1 primer:template molar ratio. The DNA/primer mixture was resuspended in 8µl water and 2µl Tris•Buffer (200 mM Tris HCl, pH 7.5, 100 mM $MgCl_2$, 250 mM NaCl) Kit). The primer and template were annealed, and chain-termination sequencing reactions were set up. A 6% sequencing gel was run at 60 watts for approximately 1 hour and 30 minutes. The gel was dried and exposed to X-ray film overnight, and the sequence determined.

EXAMPLE 6

SPNE-pIII-$(His)_6$Fusions

The HIV/pIII fusion was expressed in E. coli using the T7 polymerase system from Rosenberg, A.H. et. al., Gene 56, 125 (1987). The plasmid pET-3a (commercially available from Novagen, Madison, WI) was digested with Xba I and BamHI and the 5 kb vector fragment isolated. The isolated vector fragment was ligated with the Xba I,Bgl II-digested HIV/pIII fusion prepared by polymerase chain reaction (PCR) of the candidate HIV fuse phage clones.

Two synthetic DNA oligomers were used to amplify a portion of the phage pIII gene (including the HIV sequences) and append sequences which permit efficient expression and purification of the pIII product. The first synthetic DNA oligomers, 5' CCCTCTAG AAATAATTTTGTTTAACTTTAAGAAGGAGATATACATATGGCC GACGGGGCT 3' (SEQ ID NO:34), has homology with the fuse phage pIII gene with sequences encoding the mature amino terminus of Ala-Asp-Gly-Ala. PCR amplification from this site incorporates sequences

encoding the mature pIII protein, and rebuilds the pET-3a vector from the Xba I site to the initiating methionine.

The second synthetic DNA oligomer, sequence 5' CTCAGATCTATTAATGGTGATGGTGATGATGTATTTTGTCACA ATCAATAGAAAATTC 3' (SEQ ID NO:35) encodes the reverse strand of the carboxyl-terminal portion of pIII ending with residues Cys-Asp-Lys-Ile (SEQ ID NO:36). PCR with this oligo rebuilds the fuse phage pIII gene up to the transmembrane domain and appends six histidine residues to the carboxyl-terminal isoleucine. The presence of the histidine residues facilitates purification of the pIII fusion protein by metal chelation chromotography [Hochuli, E. et al., J. Chromat. 411, 177 (1987)] using nitrilotriacetic acid (NTA) resin (available from Qiagen, Chatsworth, CA).

Expression of the pIII fusion is obtained by transforming the expression plasmid into E. coli strain BL21 (DE3) [Rosenberg, A.H. et al., supra; U.S. Patent 4,952,496; Steen, et al., EMBO J 5, 1099 (1986).] This strain contains the T7 phage RNA polymerase gene under control of the lac operator/promoter. Addition of isopropylthiogalactoside (IPTG) at culture OD_{600}=0.6-0.8 induces T7 RNA polymerase expression which transcribes pIII mRNA to high levels. This RNA is translated yielding pIII fusion protein which is harvested 3-4 hours post-induction and chromatographed on NTA resin.

EXAMPLE 7

Synthesis of Selected Oligopeptide

The oligopeptide DKDKWASLDARTQMW (SEQ ID NO:1, hereinafter 1) is selected for immunological characterization. It is synthesized by the solid-phase method.

EXAMPLE 8

Extraction and Purification of OMPC

A. First Method

All materials, reagents and equipment were sterilized by filtration, steam autoclave or ethylene oxide, as appropriate; asceptic technique was used throughout.

A 300 gm (wet weight) aliquot of 0.5% phenol inactivated cell paste of Meningococcal group B11 was suspended in 1200 mls of distilled water then suspended by stirring magnetically for 20 minutes at room temperature. The suspended cells were pelleted at 20,000 xg for 45 minutes at 5°C.

For extraction, the washed cells were suspended in 1500 mls 0.1 M Tris, 0.01 M EDTA Buffer pH 8.5 with 0.5% sodium deoxycholate (TED Buffer) and homogenized with a 500 ml Sorvall omnimixer at setting 3 for 60 seconds. The resulting suspension was transferred to ten Erlenmeyer flasks (500 ml) for extraction in a shaking water bath for 15 minutes at 56°C. The extract was centrifuged at 20.000 x g for 90 minutes at 5°C and the viscous supernatant fluid was decanted (volume = 1500 mls). The decanted fluid was very turbid and was recentrifuged to clarify further at 20,000 x g for 90 minutes at 5°C. The twice spun supernatant fluid was stored at 5°C. The extracted cell pellets were resuspended in 1500 mls TED Buffer. The suspension was extracted for 15 minutes at 56°C and recentrifuged at 20,000 x g for 90 minutes. The supernatant fluids which contained purified OMPC were decanted (volume = 1500 mls) and stored at 5°C.

B. Second Method

All material, reagents, equipment and filters were sterilized by heat, filtration or ethylene oxide. One exception was the K-2 ultracentrifuge which was sanitized with a 0.5% formalin solution. Laminar flow canopies provided sterility protection during equipment connections. Aseptic techniques were followed throughout the entire

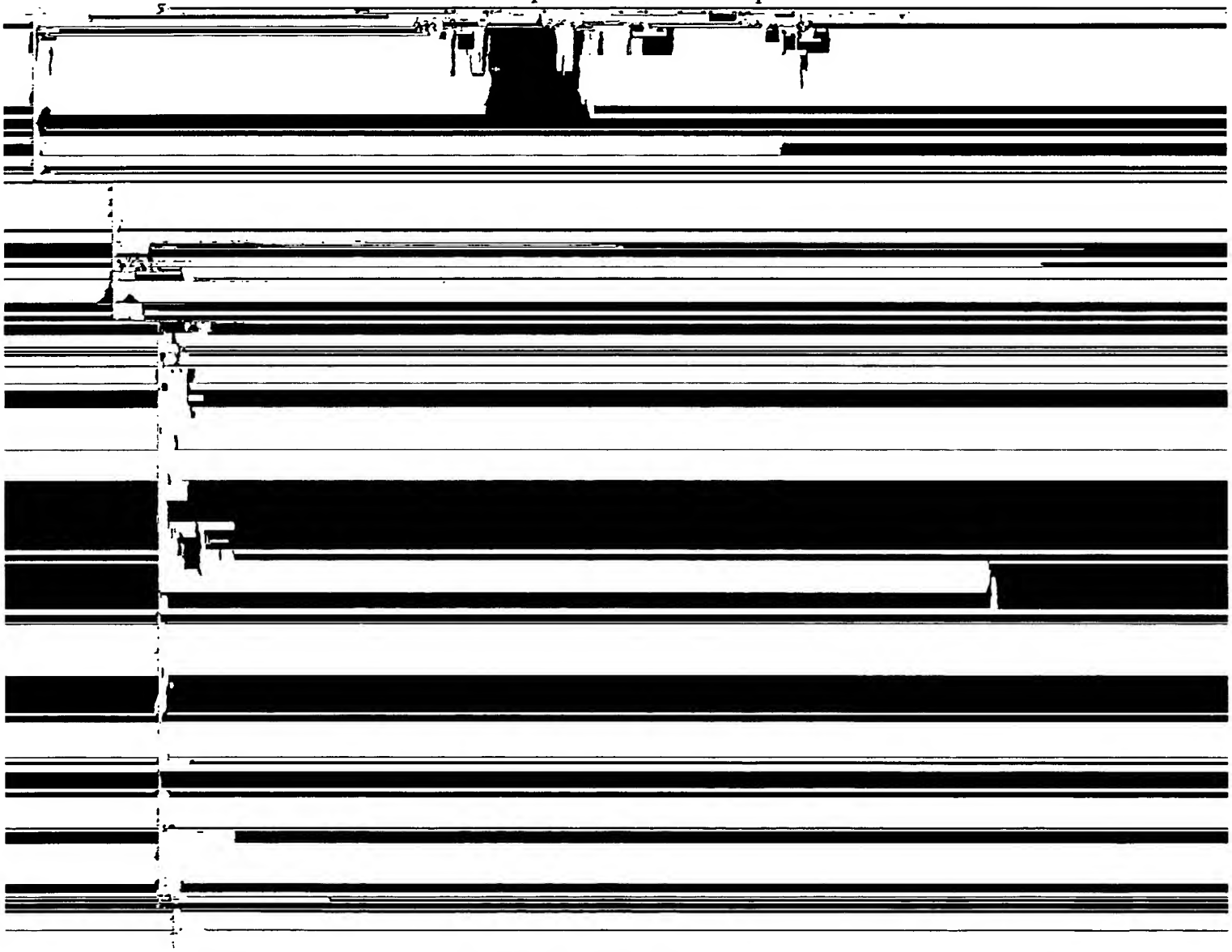
operations. Overnight storage of the protein was at 2-8°C between steps. A 0.2 micron sterile filtration was conducted just before the final diafiltration to ensure product sterility.

Two 600-liter batches of Neisseria meningitidis were fermented and killed with 0.5% phenol, then concentrated to roughly 25 liters using two 10 ft^2 0.2 micron polypropylene cross-flow filtration membranes. The concentrated broth then was diafiltered with 125 liters of cell wash buffer (0.11 M Sodium Chloride, 17.6 mM Sodium Phosphate Diabasic, 23.3 mM Ammonium Chloride, 1.34 mM Potassium Chloride, adjusted to pH 7 with 85% Phosphoric Acid followed by 2.03 mM Magnesium Sulfate Heptahydrate).

For extraction, an equal volume of 2X-TED buffer (0.2M Tris, 0.02M EDTA adjusted to pH 8.5 with concentrated HCl followed by the addition of 1.0% sodium deoxycholate) was added to the cell slurry. The resulting slurry was heated to 56% 3°C and maintained at this temperature for 30 minutes to complete the extraction of OMPC from the cells.

For further purification, the extracted cell slurry was centrifuged at 30,000 x g (18,000 rpm) in a "one-pass" flow mode in a K-ultracentrifuge, and the supernatant stream was collected. The low-speed supernatant was concentrated to 10 liters on two 0.1-micron polysulfone autoclavable hollow-fiber membranes and collected in an 18 liter sterile bottle. The filtration equipment was given two 4-liter rinses with TED buffer (0.1M Tris, 0.01M EDTA, adjusted to pH 8.5 with concentrated HCl, followed by the addition of sodium deoxycholate to 0.5%) which was combined with the retentate. The retentate was subdivided into two or three equal parts. Each part was centrifuged at 80,000 x g (35,000 rpm) for 30 minutes. The OMPC protein was pelleted, and the majority of soluble proteins, nucleic acids and endotoxins remained in the supernatant. The supernatant was discarded. The pelleted protein was resuspended by recirculating 55% 5°C TED buffer through the rotor. The first high-speed resuspensions were combined and subjected to a second low-speed spin. The second low-speed spin ensured that residual cell debris was removed from the

product stream. The second low speed supernatant was subdivided into two or three equal parts. Each fraction was given two consecutive high-speed spins. All high-speed spins were operated under the same conditions and each further purified the OMPC protein.



and filtered through a 0.2 micron cellulose acetate filter. When all fractions were permeated, an 8 L TED buffer rinse was used to flush the filtration system. The permeate and rinse were combined and concentrated to 3 liters on a 0.1 micron polysulfone autoclavable hollow fiber membrane. The material then was diafiltered with 15 liters of sterile pyrogen free water. The retentate was collected in a 4-liter bottle along with a 1-L rinse to give the final product. The final aqueous suspension was stored at 2-8°C, as purified OMPC.

transferred to a centrifuge tube and topped with pH 8.0, 0.1 M phosphate buffer (approximately 4.5 mL). The protein was pelleted via ultracentrifugation, resuspended (after homogenization) in pH 8.0, 0.1 M phosphate buffer, and repelleted by ultracentrifugation. This pellet was resuspended in 1X TED buffer, wtih a total resuspension volume of 7.0 mL. An Ellman's analysis on this solution (100 µL) revealed that it contained 0.961 mmol SH/mL solution (6.72 µmol SH total, 0.155 µmol SH/mg OMPC used).

B. Conjugation:

The beta-maleimidopropionyl peptide (5.8 mmol) is dissolved in acetonitrile (1.0 mL) giving Solution P. A solution of beta-maleimidopropionic acid (5.5 mmol) in water (1.0 mL) is prepared, which is Solution M.

Thiolated OMPC (6.0 mL, 5.77 µmol), which is prepared in step A, is transferred to a sterile 15 mL centrifuge tube. This solution is vortexed and solution M (420 mL, 2.31 µmol) added. The mixture is stirred briefly and allowed to age at room temperature (10 min). Next, the reaction mixture is vortexed and solution P (596 mL, 3.46 mmol) added. The reaction mixture is vortexed briefly and allowed to age at room temperature for 2 h.

The conjugate is spun in a clinical centrifuge to remove any precipitated material. The supernatant is removed and the conjugate is pelleted by ultracentrifugation (43K rpm, 2 h, 4°C). The pellet is resuspended in TED buffer (total volume 6.5 mL), affording 1-OMPC conjugate.

EXAMPLE 10

Immunization Protocol for 1-OMPC conjugate

Four New Zealand white rabbits (2 to 2.5 kg) are immunized with the peptide 1-OMPC conjugate vaccine (the vaccine) in the following manner: For time zero inoculations the vaccine is formulated into complete Freund's adjuvant (CFA) [1:1(v/v) of CFA

and 600 μg/ml of conjugate in saline]. Each dose (1.0 ml) consists of a total of 300 μg of vaccine. Each rabbit is inoculated with the vaccine preparation at two sites, by intra-muscular (im) injection, in the upper hind leg. Two booster inoculations are given to each rabbit at week 4 and week 8 post initial injection. The vaccine for these booster injections is formulated into incomplete Freund's adjuvant. Each dose also consists of a total of 300 μg of vaccine.

Each rabbit is bled and sera is prepared by standard methods for anti-peptide ELISA tests (Example 11A) and anti-HIV neutralization tests (Example 12). Sera collected represent time zero and biweekly intervals through week 14.

EXAMPLE 11A

Measurement of Antibody Responses in Rabbits Immunized with 1-OMPC Conjugate Vaccine (ELISA)

Elicited anti-peptide antibody responses in vaccinated rabbits are determined by the use of an enzyme-linked immuno-adsorbent assay (ELISA). In this assay, microtiter plates are coated with about 0.5 mg peptide 1 per well using an overnight incubation of peptide solution at 36°C in a humidified atmosphere.

For ELISA tests, titers are determined with 0 time and weeks 2, 4, 6, 8, 10, 12 and 14 sera. Test sera are diluted 5-fold serially, are reacted for 1 hr with the peptide adsorbed wells, and are washed extensively. Positive results are identified after reactions of phosphatase-conjugated goat anti-rabbit sera with each well for 1 hr at 36°C, washing and the addition of a solution of 1.0 mg/mL p-nitrophenyl phosphate (pNPP) in 10% diethanolamine, 0.5 mM $MgCl_2$ (pH 9.8) to each well. This last reaction proceeds for 30 minutes at room temperature and is stopped by addition of 3.0 N NaOH. Absorbance at 405 nm is determined by using a plate reader.

EXAMPLE 11B

Preparation of Human Peripheral Blood Mononuclear Cells (PBMC):

Human PBMC cultures were prepared from fresh HIV (-) donor buffy coat cells. The cells were purified in Ficoll Hypaque gradients followed by collecting and washing the PBMC fraction. These cells were cultured in RPMI 1640 supplemented with 20% fetal bovine serum and were stimulated with phytohemagglutinin at 5 μg/ml. After 48 h, the cultures were expanded with additional medium and were supplemented with IL-2 at a final concentration of 40 units/ml. After three more days the cultures were centrifuged and cells resuspended in fresh RPMI 1640 supplemented with 20% fetal bovine serum and 40 units/ml IL-2. Cells prepared in this manner were used for making primary isolate virus stocks and in antibody-mediated virus infectivity reduction tests.

EXAMPLE 12

Measurement of Virus Neutralizing Antibody Responses Elicited in Rabbits Immunized with 1-OMPC Conjugates.

Neutralization of Infectivity in MT-4 Cells in vitro: For neutralization tests 2-fold serial dilutions of sera are made and 100 μL volumes are used in each test well in 96 well culture plates. All sera are heat-inactivated before use. Generally 1:10 is the starting dilution of sera. An aliquot of 100 μL virus stock dilution is added to each test well. The virus-antisera mixtures are incubated at 37°C for 1 hr after which 1 x 10^4 MT-4 cells in 50 μL of culture medium are added to each well and the cultures are incubated for 7 days. The level of neutralization is determined by using the MTT dye reduction readout. MTT is added to each well to 500 μg/mL, incubated at 37°C for 2 hr, and solubilized after addition of acid-isopropanol (0.04N HCl in isopropanol) to approximately 50% of the volume of each well. A clearly distinguishable bluish-purple color develops in wells containing viable cells that are protected from infection due to virus neutralization

by anti-peptide 1 antibody whereas wells containing MT-4 cells killed by the infection remain yellow. The neutralization endpoints are determined as the last dilution of antisera preparation that prevents cell killing. Uninfected MT-4 cells are cultured with each test and a virus retitration is performed with each analysis.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: J.A. Kessler, A.J. Conley, B.A. Arnold

(ii) TITLE OF INVENTION: Selected Principal Neutralization Epitopes for Antibody that Neutralizes HIV in Peripheral Blood Mononuclear Cells

(iii) NUMBER OF SEQUENCES: 40

(iv) CORRESPONDENCE ADDRESS:
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(F) ZIP: 07065

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY AGENT INFORMATION:
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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) IMMEDIATE SOURCE: Random Epitope Library Alpha

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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Asp Lys Asp Lys Trp Ala Ser Leu Asp Ala Arg Thr Gln Met Trp
1               5                  10                  15
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(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) IMMEDIATE SOURCE: Random Epitope Library Alpha

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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Glu Asn Ala Ile Asp Lys Trp Ala Thr Leu Tyr Trp Ala Tyr Gly
1               5                  10                  15
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) IMMEDIATE SOURCE: Random Epitope Library Alpha

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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Ala Phe Met Thr Val Asp Lys Trp Ala Met Phe Trp Pro Thr Gln
1               5                  10                  15
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) IMMEDIATE SOURCE: Random Epitope Library Alpha

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

His Met Ser Pro Phe Asp Lys Trp Glu Phe Ile Thr Ala Arg Arg
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) IMMEDIATE SOURCE: Random Epitope Library Alpha

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Ser Leu Phe Ser Glu Asp Lys Trp Ser Tyr Leu His Ser Asn Ala
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) IMMEDIATE SOURCE: Random Epitope Library Alpha

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met Ser His Tyr Asp Ile Asp Lys Trp Gly Gly Met Thr Ser Gln
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) IMMEDIATE SOURCE: Random Epitope Library Alpha

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Thr Pro Asp Gly Thr Leu Phe Phe Asp Lys Trp Ser Leu Phe Arg
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) IMMEDIATE SOURCE: Random Epitope Library Alpha

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Pro Lys Met Gly Ser Leu His Met Asn Asp Lys Trp Val Ser Val
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) IMMEDIATE SOURCE: Consensus Peptide A

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Ser Leu Xaa Asp Phe Asp Lys Trp Ala Ser Leu Thr Ser Arg
1 5 10

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) IMMEDIATE SOURCE: Random Epitope Library Alpha

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Asp Asp Leu Asp Arg Trp Gly Arg Thr Asp Trp Asn Phe Phe Leu
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) IMMEDIATE SOURCE: Random Epitope Library Alpha

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Glu Asp Val Leu Asp Arg Trp Val Leu Leu Asp His Trp Asp Ser
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) IMMEDIATE SOURCE: Random Epitope Library Alpha

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Gly Gln Thr Ala Ala Asp Leu Asp Lys Trp Tyr Val Gly Gly Asp
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) IMMEDIATE SOURCE: Random Epitope Library Alpha

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Xaa Pro Leu Pro Ser Asp Leu Asp Lys Trp Glu Phe Leu Asn Arg
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) IMMEDIATE SOURCE: Random Epitope Library Alpha

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Val Val Asn Ser Leu Pro Leu Asp Arg Trp Ala Tyr Ser Tyr Glu
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) IMMEDIATE SOURCE: Consensus Peptide B

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Asp Asp Leu Asp Arg Trp Xaa Xaa Leu Asp
1 5 10

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) IMMEDIATE SOURCE: Random Epitope Library Alpha

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Met Lys Glu Asp Asp Lys Trp Val Pro Val Lys Gly Arg Lys Phe
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) IMMEDIATE SOURCE: Random Epitope Library Alpha

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Met Leu Glu Gly Asp Lys Trp Ser Asp Ile Gly Met Val Phe Val
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) IMMEDIATE SOURCE: Random Epitope Library Alpha

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Arg Gly Arg Ala Asp Glu Pro Asp Arg Trp Ala Leu Met Phe Asp
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) IMMEDIATE SOURCE: Random Epitope Library Alpha

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Thr Ala Asn Ser His Trp Leu Glu Tyr Asp Arg Trp Ser Glu Val
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) IMMEDIATE SOURCE: Consensus Peptide C

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Met Leu Glu Xaa Asp Lys Trp Ser
1 5

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) IMMEDIATE SOURCE: Consensus Peptide D

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Met Leu Glu Xaa Asp Arg Trp Ser
1 5

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) IMMEDIATE SOURCE: Random Epitope Library Alpha

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

Glu Leu Asp Arg Trp Ala Glu Leu Glu Met Arg Gly His Leu Asn
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) IMMEDIATE SOURCE: Random Epitope Library Alpha

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

Glu Leu Asp Lys Trp Gly Trp Met Ala Ser His Glu Ala His Ile
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) IMMEDIATE SOURCE: Random Epitope Library Alpha

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

Val Phe Glu Leu Asp Lys Trp Ser Gly Arg Asp Glu Glu Trp Ala
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) IMMEDIATE SOURCE: Consensus Peptide E

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

Glu Leu Asp Lys Trp Xaa Xaa Xaa Xaa Xaa Xaa Xaa Ala
1 5 10

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iv) ANTI-SENSE: NO

(vi) IMMEDIATE SOURCE: Library Alpha formula

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

Ala Asp Gly Ala Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
1 5 10 15

Xaa Xaa Xaa Xaa Gly Ala Ala Gly Ala
20

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iv) ANTI-SENSE: NO

(vi) IMMEDIATE SOURCE: Library BETA formula

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

Ala Asp Gly Ala Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Gly Pro
1 5 10 15

Xaa Arg Xaa Xaa Gly Ala Ala Gly Ala
20

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iv) ANTI-SENSE: NO

(vi) IMMEDIATE SOURCE: Library GAMMA formula

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

Ala Asp Gly Ala Leu Leu Xaa Xaa Xaa Xaa Xaa Gly Pro Xaa Arg
1 5 10 15

Xaa Xaa Xaa Xaa Xaa Leu Leu Gly Ala Ala Gly Ala
20 25

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iv) ANTI-SENSE: NO

(vi) IMMEDIATE SOURCE: Library DELTA formula

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

Ala Asp Gly Ala Cys Xaa Xaa Xaa Xaa Xaa Gly Pro Xaa Arg Xaa
1 5 10 15

Xaa Xaa Xaa Xaa Cys Gly Ala Ala Gly Ala
20

2 INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iv) ANTI-SENSE: NO

(vi) IMMEDIATE SOURCE: Library EPSILON formula

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

Ala Asp Gly Ala Cys Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
1 5 10 15

Xaa Xaa Xaa Xaa Xaa Cys Gly Ala Ala Gly Ala
20 25

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA primer

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

GTAAATGAAT TTTCTGTATG AGG
23

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA primer

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

TCGAAAGCAA GCTGATAAAC CG
22

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA primer

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

ACAGACAGCC CTCATAGTTA GCG
23

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 60 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA primer

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

CCCTCTAGAA ATAATTTTGT TTAACTTTAA GAAGGAGATA TACATATGGC CGACGGGGCT
60

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 58 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA primer

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

CTCAGATCTA TTAATGGTGA TGGTGATGAT GTATTTTGTC ACAATCAATA GAAAATTC
58

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: carboxy terminal fragment of pIII
internal to fusion peptide

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

Cys Asp Lys Ile
1

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: amino terminal flank for SPNE

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

Ala Asp Gly Ala
1

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: carboxy terminal flank for SPNE

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

Gly Ala Ala Gly Ala
1 5

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) IMMEDIATE SOURCE: Consensus peptide for SEQ. ID. No.: 1-8, 10-14, 16-19, 22-24

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

Ser Met Leu Glu Leu Asp Lys Trp Ala Leu Leu Xaa Xaa Arg Phe
1 5 10 15

Xaa Leu

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) IMMEDIATE SOURCE: Consensus Peptide F

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

Ser Met Leu Glu Leu Asp Lys Trp Ala Leu Leu Xaa Xaa Arg Phe
1 5 10 15

Xaa Leu

WHAT IS CLAIMED IS:

1. An antigenic conjugate of HIV-specific, selected principal neutralization epitopes covalently linked to purified outer membrane proteosome of Neisseria, wherein said conjugate is of the formula

$$(SPNE)_n\sim(OMPC)$$

wherein:

SPNE	is the selected principal neutralization epitope of HIV, which is a polypeptide of one or more amino acid sequences of Table A or fragment thereof, said fragment having at least 5 amino acids in length and including DRW or DKW in its sequence;
n	indicates the number of polypeptides of SPNE covalently linked to OMPC and is 1-200;
~	indicates covalent linkage;
OMPC	is purified outer membrane proteosome of Neisseria, said conjugate optionally substituted with a^-, which is an anion or polyanion at physiological pH, said a^- consisting of one to five residues of anions selected from the group consisting of carboxylic, sulfonic, propionic or phosphonic acid,

or pharmaceutically acceptable salt thereof.

2. The antigenic conjugate of Claim 1 wherein the covalent linkage between SPNE and OMPC consists essentially of a bigeneric spacer.

3. The antigenic conjugate of Claims 1 or 2, wherein said OMPC is derived from Neisseria meningitidis.

4. An AIDS vaccine comprising an antigenic conjugate of HIV-specific selected principal neutralization epitopes having one or more of the sequences of Table A, said epitopes covalently linked to purified outer membrane proteosome of Neisseria, said conjugate mixed with a suitable immunological adjuvant, carrier or vector, said vaccine to be used pre- and post-exposure to prevent or treat HIV infection or disease, said vaccine capable of eliciting specific HIV neutralizing antibodies, said purified outer membrane proteosome optionally substituted with a^-, which is an anion or polyanion at physiological pH, said a^- consisting of one to five residues of anions selected from the group consisting of carboxylic, sulfonic, propionic or phosphonic acid.

5. An AIDS vaccine of Claim 4 wherein the covalent linkage between SPNE and OMPC consists essentially of a bigeneric spacer.

6. An AIDS vaccine of Claim 4 wherein said OMPC is derived from Neisseria meningitidis.

7. A pharmaceutical composition comprising an antigenic conjugate of HIV-specific selected principal neutralization epitopes having one or more of the sequences of Table A, said epitopes covalently linked to purified outer membrane proteosome of Neisseria, said conjugate mixed with a suitable immunological adjuvant, said composition useful as a vaccine capable of producing specific HIV neutralizing antibody in mammals, said purified outer membrane proteosome optionally substituted with a^-, which is an anion or polyanion at physiological pH, said a^- consisting of one to five residues of anions selected from the group consisting of carboxylic, sulfonic, propionic or phosphonic acid.

8. The pharmaceutical composition of Claim 7 wherein the covalent linkage between SPNE and OMPC consists essentially of a bigeneric spacer.

9. The pharmaceutical composition of Claim 7 wherein said OMPC is derived from Neisseria meningitidis.

10. A method of vaccinating against ARC or AIDS, comprising administering an effective amount of a pharmaceutical composition comprising an antigenic conjugate of HIV-specific selected principal neutralization epitopes having one or more of the sequences of Table A, said epitopes covalently linked to purified outer membrane proteosome of Neisseria, said conjugate mixed with a suitable immunological adjuvant, said purified outer membrane proteosome optionally substituted with a^-, which is an anion or polyanion at physiological pH, said a^- consisting of one to five residues of anions selected from the group consisting of carboxylic, sulfonic, propionic or phosphonic acid.

11. A method of preventing infection by HIV, comprising administering an effective amount of a pharmaceutical composition comprising an antigenic conjugate of HIV-specific selected principal neutralization epitopes having one or more sequences of Table A, said epitopes covalently linked to purified outer membrane proteosome of Neisseria, said conjugate mixed with a suitable immunological adjuvant, said purified outer membrane proteosome optionally substituted with a^-, which is an anion or polyanion at physiological pH, said a^- consisting of one to five residues of anions selected from the group consisting of carboxylic, sulfonic, propionic or phosphonic acid.

12. A method of treating AIDS, comprising administering an effective amount of a pharmaceutical composition comprising an antigenic conjugate of HIV-specific selected principal neutralization epitopes having one or more sequences of Table A, said epitopes covalently linked to purified outer membrane proteosome of Neisseria, said conjugate mixed with a suitable immunological adjuvant, said purified outer membrane proteosome optionally substituted with a^-, which is an anion or polyanion at physiological pH, said a^- consisting of

one to five residues of anions selected from the group consisting of carboxylic, sulfonic, propionic or phosphonic acid.

13. A method of treating infection by HIV, comprising administering an effective amount of a pharmaceutical composition comprising an antigenic conjugate of HIV-specific selected principal neutralization epitopes having one or more sequences of Table A, said epitopes covalently linked to purified outer membrane proteosome of Neisseria, said conjugate mixed with a suitable immunological adjuvant, said purified outer membrane proteosome optionally substituted with a^-, which is an anion or polyanion at physiological pH, said a^- consisting of one to five residues of anions selected from the group consisting of carboxylic, sulfonic, or propionic phosphonic acid.

14. HIV-specific selected principal neutralization epitope polypeptides having any of sequences of Table A.

15. HIV-specific selected principal neutralization consensus polypeptide having any of the sequences of Table A.

Patents Act 1977 Examiner's report to the Comptroller under Section 17 (The Search report) -14-	Application number GB 9419255.6
Relevant Technical Fields (i) UK Cl (Ed.M) C3H (HHX2) (ii) Int Cl (Ed.5) A61K 39/21, 39/095, 39/385	Search Examiner MR C SHERRINGTON Date of completion of Search 5 DECEMBER 1994
Databases (see below) (i) UK Patent Office collections of GB, EP, WO and US patent specifications. (ii) ONLINE DATBASES: WPI, CLAIMS, DIALOG/BIOTECH	Documents considered relevant following a search in respect of Claims :- 1 TO 9

Categories of documents

X: Document indicating lack of novelty or of inventive step.

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A: Document indicating technological background and/or state of the art.

P: Document published on or after the declared priority date but before the filing date of the present application.

E: Patent document published on or after, but with priority date earlier than, the filing date of the present application.

&: Member of the same patent family; corresponding document.

Category	Identity of document and relevant passages		Relevant to claim(s)
P,A	GB 2271995 A	(MERCK & CO INC) whole document, especially Examples 31, 32; Claim 10, SEQ ID 6 and 7	1 to 9
A	EP 0402088 A2	(MERCK & CO INC) especially page 7, lines 38 to 40; Claims 8, 26	1 to 9
A	EP 0519554 A1	(MERCK & CO INC) whole document especially SEQ ID Number 22, page 51; Example 18; Claims 95, 11e	1 to 9

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